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Please
PATENT entered

5/26/04

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Robert Michael ROBERTS Ex
Jonathan Andrew GREEN and
Sancai XIE

Serial No.: 09/273,164

Filed: March 19, 1999

For: COMPOSITIONS AND METHODS FOR EARLY PREGNANCY DIAGNOSIS

Group Art Unit: 1643

Examiner: C. Cheu

Atty. Dkt. No.: UVMO:003

CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on the date below:

Date Robert E. Hanson

DECLARATION OF JONATHAN A. GREEN UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, JONATHAN A. GREEN, HEREBY DECLARE AS FOLLOWS:

1. I am a co-inventor of the subject matter disclosed and claimed in the above-referenced patent application.

2. I am currently employed by The University of Missouri as an Assistant Professor. I hold a Ph.D. in Biochemistry from the University of Missouri. I have been conducting research in the area of biochemistry and reproductive biology since 1991.

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3. I previously supplied a Declaration in this patent application submitting data demonstrating the isolation and use of monoclonal antibodies that detect PAGs disclosed in the above-referenced patent application. The data presented at that time demonstrated that PAGs 4, 6, 7, 16 and 20 are absent about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals. I am submitting this Declaration to present further data obtained since the time of the studies described in my previous Declaration. This data demonstrates that, in addition to the PAGs listed above, PAG 17 and PAG 21 are also undetectable at about two months post-partum.

4. *Identification of PAGs bound by L4, A6 and J2 monoclonal antibodies.*

The isolation of monoclonal antibodies L4, A6, and J2 was as described in my previous Declaration. Further studies were carried out under my supervision to identify the PAGs detected by these antibodies as follows:

One mg of each purified mAb was first crosslinked to 2 mL of matrix in the ImmunoPure Protein A IgG Plus Orientation kit (Pierce Biotechnology, Inc. Rockford, IL, USA) by following the manufacturer's instructions. Cotyledonary extracts were collected from 18 cm and 40 cm crown-rump bovine fetuses, dialyzed against 2000 volumes of binding buffer and 100 mg of total protein from each extract was applied separately to each matrix. The columns were washed in binding buffer until the absorbance of the flow-through at 280 nm was at baseline. The bound protein was eluted from the column with 20mM sodium formate, pH 2.8. The eluted proteins were subjected to SDS-PAGE followed by in-gel trypsin digestion, reduction and alkylation of cysteines. The masses of the resulting peptides were then determined by Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry on a Voyager-DE™ PRO Biospectrometry Workstation (Applied Biosystems, Foster City, CA, USA). The monoisotopic masses in the acquired spectra were used for searching against a nonredundant translated mammalian sequence database (NCBInr) by using the Protein Prospector MS FIT program (http://prospector.ucsf.edu/).

The A6 monoclonal antibody exhibited the greatest ability to bind PAG in the placental extracts. The eluted material migrated at three distinct relative molecular mobilites on SDS-PAGE: 55,000, 65,000 and 75,000. Peptide mass fingerprinting revealed that the 75kDa band

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consisted predominantly of PAG7 and PAG6 and the 55kDa band consisted predominantly of PAG16 and PAG4, although weak matches were also observed for PAG17, PAG20 and PAG21 in this band (Table 1). The 65kDa band did not produce many peptides amenable to fingerprinting, but the few that were produced were found to match PAG7.

The yields from the J2 and L4 affinity columns were not as robust as those from the A6 matrix, however, both did permit the purification of a ~70,0000 Da protein. The J2-purified protein did not produce many tryptic fragments, but those that were produced matched PAG20 (Table 2). The L4-purified protein was more easily digested and produced numerous masses for fingerprinting. The main PAG isolated from the extracts was PAG21 although other PAGs (PAG17, PAG16 and PAG20) were clearly represented, albeit at lower concentrations (Table 3). The A6 and L4 antibodies bound PAG17 with lower affinity, but the results confirmed the undetectability of this PAG at about two-months post-partum. The major PAGs recognized by each of the monoclonal antibodies (and their relationship to other bovine PAGs) are indicated in the neighbor-joining tree (FIG. 1).

5. The result of the studies demonstrated that PAGs 4, 6, 7, 16, 17, 20 and 21 are undetectable about two-months post-partum and that antibodies for these PAGs may be used in assays for the detection of pregnant bovine animals.

6. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

21 May 2004
Date

Jonathan A. Green

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Table 1. Assignment of tryptic digest fragments of PAG affinity purified with the A9 monoclonal antibody.

75kDa protein

98 kDa protein*

Table 2. Assignment of tryptic digest fragments of PAG affinity purified with the J2 monoclonal antibody.

BOS TAURUS. (AF192337) pregnancy-associated glycoprotein-20

m/z submitted	MH+ matched	Delta ppm	Start	Peptide Sequence	Modifications
1048.599	1048.5311	62.4526	352-359	(R)LYFSVFDR(G)	
1667.861	1667.8607	0.2052	29-42	(R)KTLSGKNMLNNFLK(E)	1PO4
1789.975	1789.9271	26.7724	123-138	(R)LTNKTFGITYGSGRMK(G)	1Met-ox
1794.882	1794.8039	43.5329	217-231	(K)GSVVMFGGVDHRYYK(G)	1PO4
1885.032	1885.0505	-9.7585	3-18	(K)WLVLLGLVAFSECIFK(I)	
1927.919	1927.9264	-3.8439	327-342	(R)AYVLKDFTGNCYTTFK(E)	
2221.269	2221.1366	59.1792	117-136	(R)QSSTFRLTNKTFGITYGSGR(M)	

Table 3. Assignment of tryptic digest fragments of PAG affinity purified with the L4 monoclonal antibody.

BOS TAURUS. (AF192336) pregnancy-associated glycoprotein-21 .

m/z submitted	MH+ matched	Delta ppm	Start	Peptide Sequence	Modifications
842.537	842.5464	12.826	282-288	(K)LVNKIQK(L)	
965.513	965.542	-30.0409	327-334	(R)AYILKDSR(G)	
970.519	970.5413	-23.0012	281-288	(R)KLVNKIQK(L)	
1032.578	1032.5155	60.5788	962-969	(R)VYFSVFDR(G)	
1088.596	1088.5378	55.4428	127-136	(K)TFSITYGSGR(M)	
1178.673	1178.6646	7.1432	327-336	(R)AYILKDSRGR(C)	
1201.61	1201.6152	-4.3171	337-345	(R)CYTARKKQR(F)	
1359.729	1359.6999	46.4076	215-228	(R)EGSVVMFGGVDHR(Y)	
1405.714	1405.6534	43.0948	216-228	(R)EGSVVMFGGVDHR(Y)	1Met-ox
1733.952	1733.8838	39.4834	113-128	(R)FRQHQSSTFRPTNK(T)	
1830.003	1829.9074	52.2487	346-361	(R)FSSSTETWLLGDAFLR(V)	
1860.005	1859.875	69.8735	216-231	(R)EGSVVMFGGVDHRYYK(G)	1Met-ox
1999.089	1998.9925	53.2888	232-248	(K)GELNWVPLIEEGDWSVR(M)	
2153.122	2153.0327	41.4774	30-47	(K)TLSGKNMLNNFLKEHGNR(L)	1PO4

Weaker matches were observed for PAG-17, PAG-18 and PAG-20

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